BLAST

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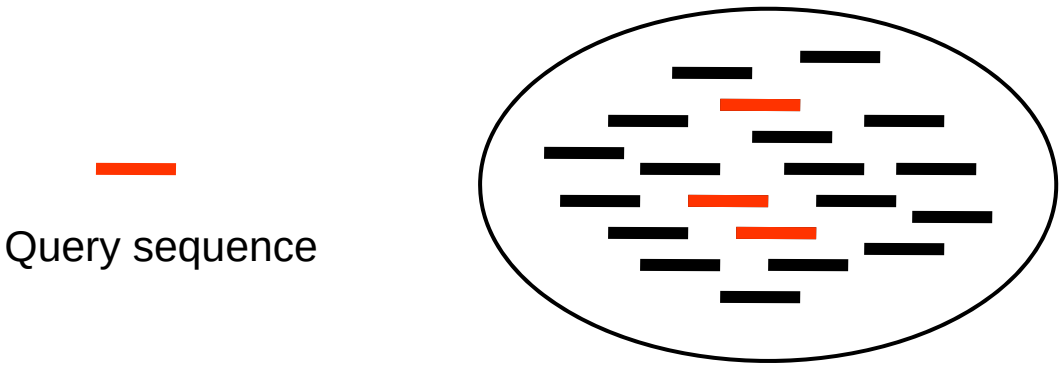
Database searching

Using pairwise alignments to search databases for similar sequences



Database searching

Most common use of pairwise sequence alignments is to search databases for related sequences. For instance: find probable function of newly isolated protein by identifying similar proteins with known function.

Most often, ***local*** alignment (“Smith-Waterman”) is used for database searching: you are interested in finding out if ANY domain in your protein looks like something that is known.

Often, full Smith-Waterman is too time-consuming for searching large databases, so heuristic methods are used (fasta, BLAST).

Database searching: heuristic search algorithms

FASTA (Pearson 1995)

Uses heuristics to avoid calculating the full dynamic programming matrix

Speed up searches by an order of magnitude compared to full Smith-Waterman

The statistical side of FASTA is still stronger than BLAST

BLAST (Altschul 1990, 1997)

Uses rapid word lookup methods to completely skip most of the database entries

Extremely fast

One order of magnitude faster than FASTA

Two orders of magnitude faster than Smith-Waterman

Almost as sensitive as FASTA

BLAST flavors

BLASTN

Nucleotide query sequence

Nucleotide database

BLASTP

Protein query sequence

Protein database

BLASTX

Nucleotide query sequence

Protein database

Compares all six reading frames with the database

TBLASTN

Protein query sequence

Nucleotide database

”On the fly” six frame translation of database

TBLASTX

Nucleotide query sequence

Nucleotide database

Compares all reading frames of query with all reading frames of the database

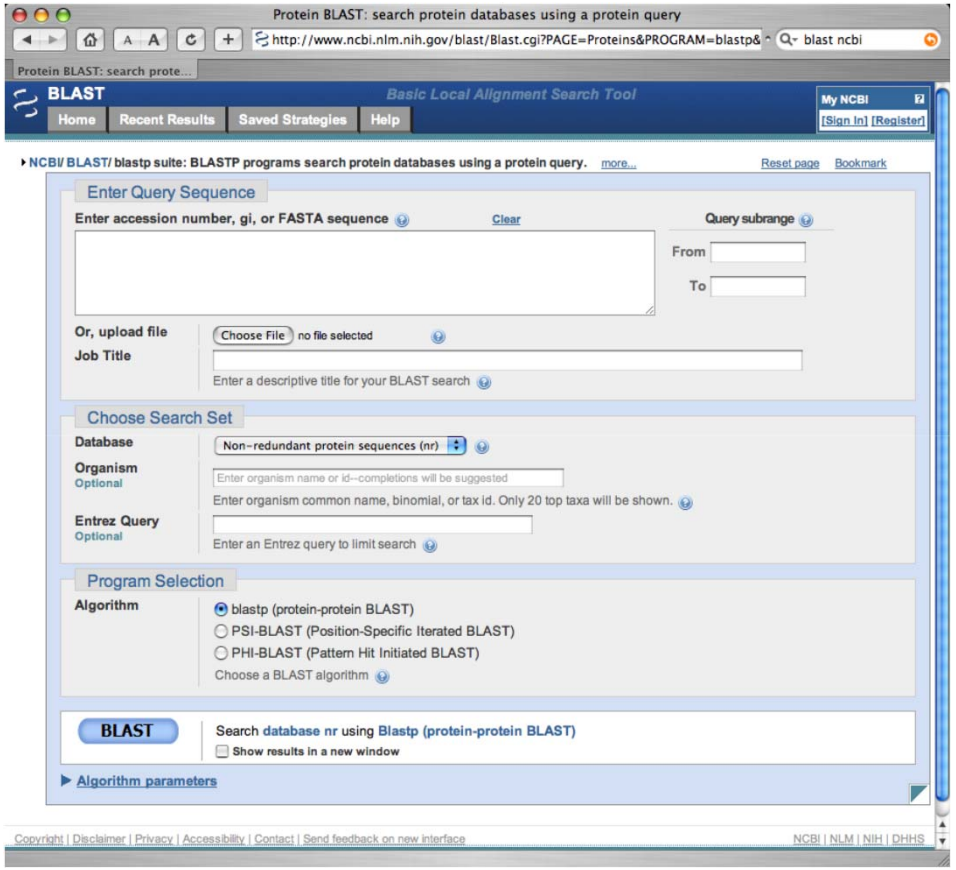
Searching on the web: BLAST at NCBI

Very fast computers dedicated to running BLAST searches

Many databases that are always up to date (e.g. NR and Human Genome)

Nice simple web interface

But you still need knowledge about BLAST to use it properly



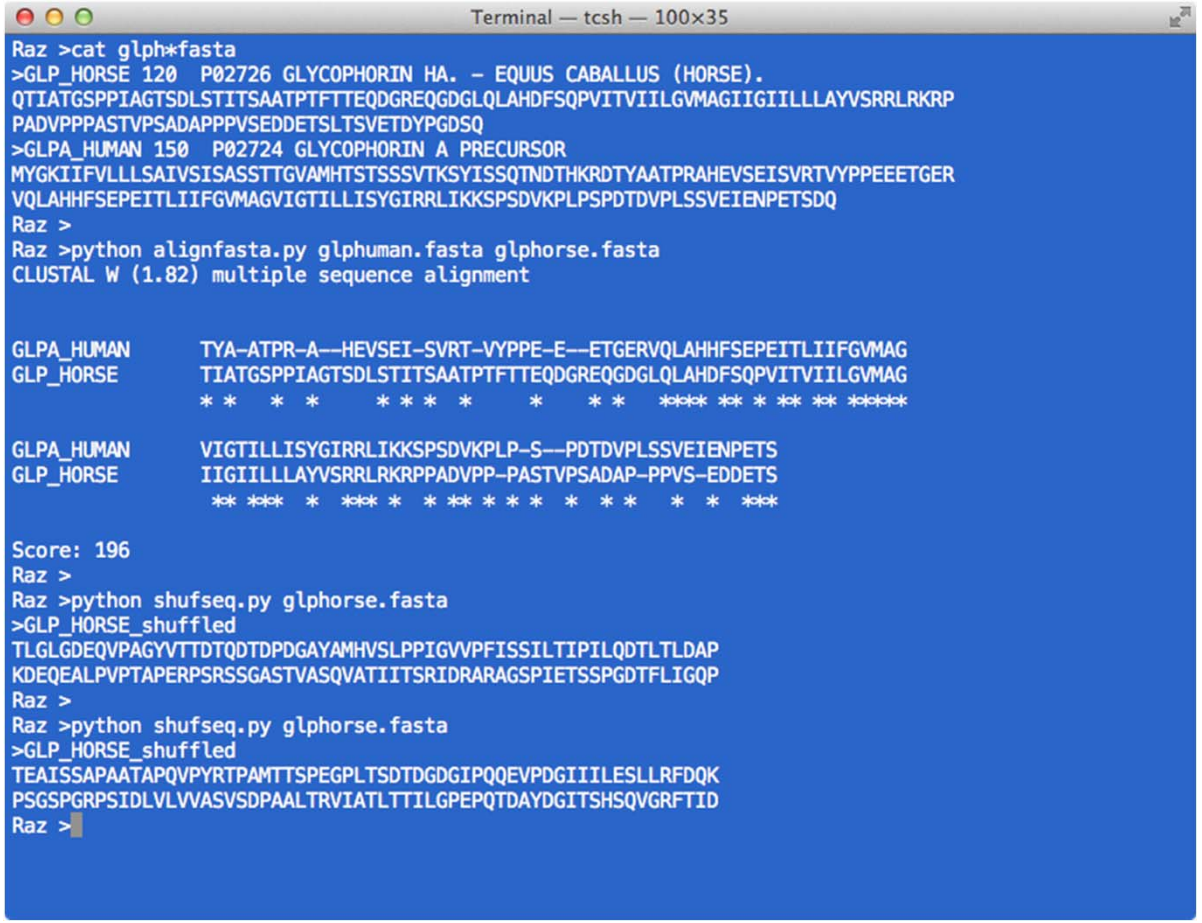
When is a database hit significant?

- **Problem**:
  - Even unrelated sequences can be aligned (yielding a low score)
  - How do we know if a database hit is meaningful?
  - When is an alignment score sufficiently high?

- **Solution**:
  - Determine the range of alignment scores you would expect to get for random reasons (i.e., when aligning unrelated sequences).
  - Compare actual scores to the distribution of random scores.
  - Is the real score much higher than you'd expect by chance?

Distribution of random alignment scores

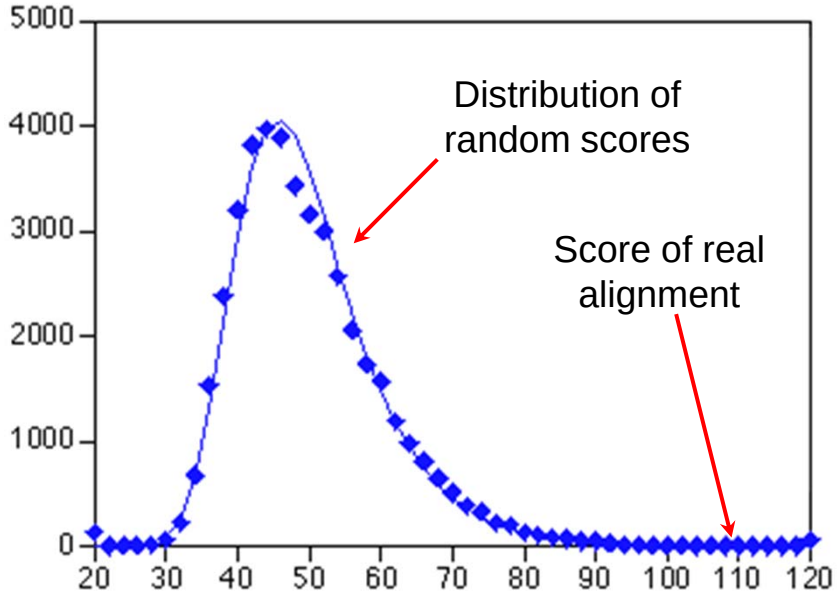
- **Software simulation**



Significance of alignment score expressed as E-value

Searching a database of **unrelated** sequences results in scores following an extreme value distribution

The exact shape and location of the distribution depends on the exact nature of the database and the query sequence



E-value: the number of **random hits** to **expect** for any given score

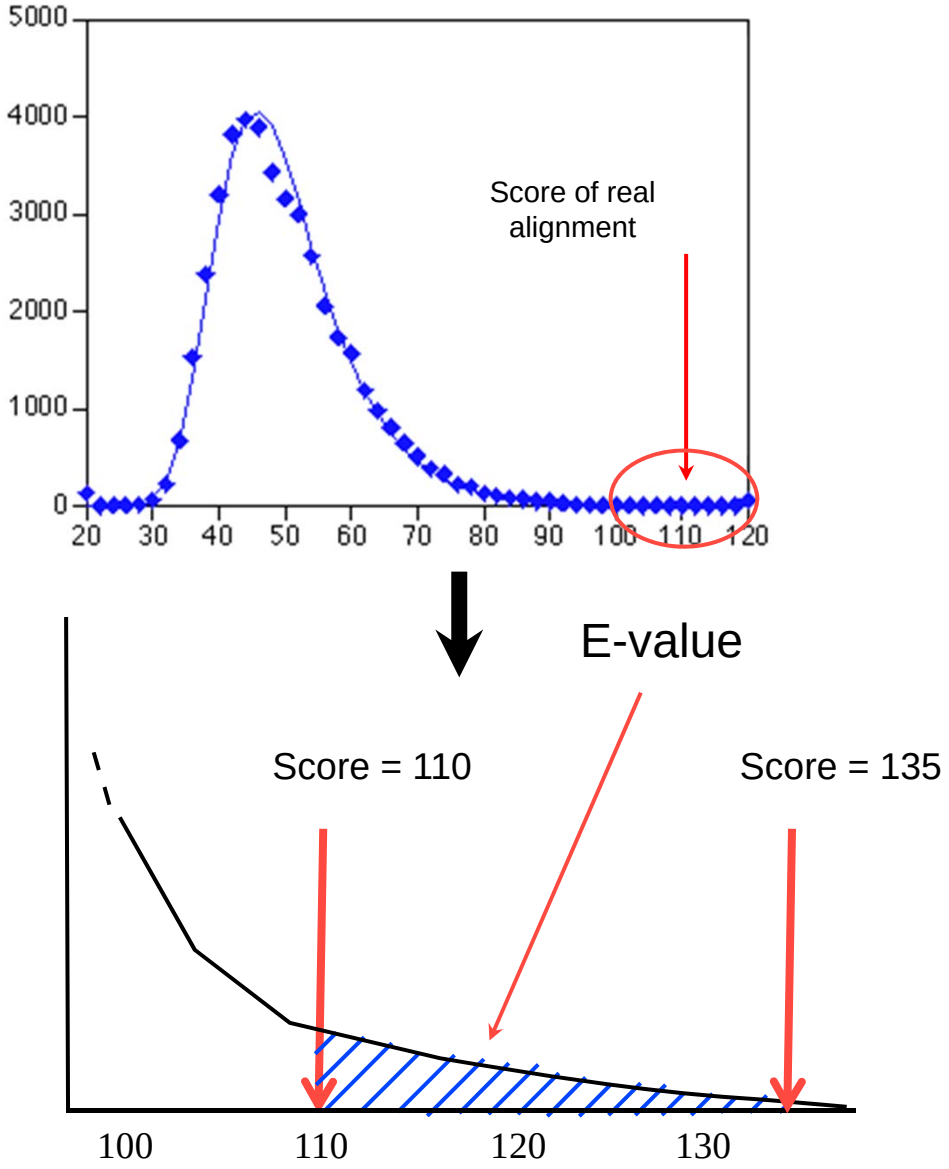
Want E-values below 1 (the lower the better)

Significance of alignment score expressed as E-value

E-value / Expect-value:
Number of unrelated hits with an equal or better alignment score to expect due to strictly stochastic reasons.

Example:
Alignment score = 110
E-value = 8.7

Alignment score = 135
E-value = 0.0001



BLAST heuristics

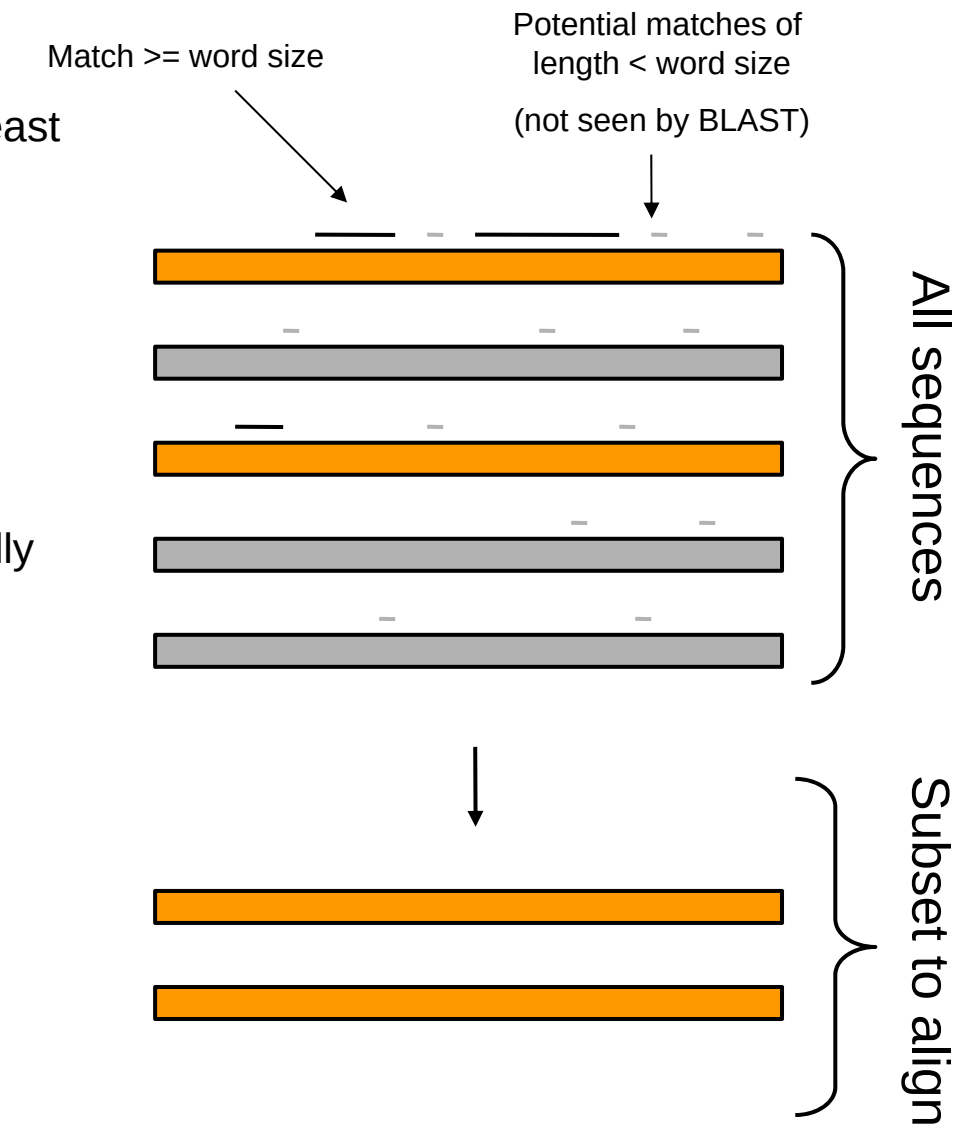
- BLAST speeds up the search >100x by pre-screening the database sequences and only performing the full Dynamic Programming on "*promising*" sequences.

- Promising sequences: database sequences that have sub-strings ("words") which also occur in the query sequence (found rapidly using a so-called "**suffix-tree**")

- **BLASTN** and **BLASTP** use different criteria for overlap required for a sequence to be deemed promising

BLASTN

- Heuristics:
  - Perfect match “word” of at least size: 7, 11 (default) or 15.
- Alignment matrix:
  - Match: **1**
  - Mismatch: **-3**
- Notice: All mismatches are equally penalized:
  - E.g. A:G == A:C == A:T
  - More advanced models for DNA evolution does exist.



BLASTP

- Heuristics:
  - 2 x “Near match” within a window.
  - Default word length: 3 aa
  - Default window length: 40 aa
- Alignment matrix:
  - PAM and BLOSUM-series (default: BLOSUM 62)
- Notice: These alignment matrices incorporate knowledge about protein evolution.